L Number	Hits	Search Text	DB	Time stamp
1	299	sulfolobus or acidocaldarius	USPAT; US-PGPUB	2001/11/09 16:07
2	3346	trehalose	USPAT; US-PGPUB	2001/11/09 16:07
3	29	(sulfolobus or acidocaldarius) and trehalose	USPAT; US-PGPUB	2001/11/09 16:11
4	98	non adj reducing adj saccharide$1	USPAT; US-PGPUB	2001/11/09 16:11
5	18	(sulfolobus or acidocaldarius) and (non adj reducing adj saccharide$1)	USPAT; US-PGPUB	2001/11/09 16:12
6	3366	$trehalose	USPAT; US-PGPUB	2001/11/09 16:12
7	29	(sulfolobus or acidocaldarius) and $trehalose	USPAT; US-PGPUB	2001/11/09 16:12
8	102	(trehalose or (non adj reducing adj saccharide$1)) near6 (synthes$8 or produc$8 or form$6) near6 enzym$8	USPAT; US-PGPUB	2001/11/09 16:19
9	89	(trehalose or (non adj reducing adj saccharide$1)) near6 (synthes$8 or produc$8 or FORM$6(FORMULAA)) near6 enzym$8	USPAT; US-PGPUB	2001/11/09 16:24
10	25	(trehalose or (non adj reducing adj saccharide$1)) near6 (synthes$8 or PRODUC$8(PRODUCT-11.1) or FORM$6(FORMULAA)) near6 enzym$8	USPAT; US-PGPUB	2001/11/09 16:26
11	102	((trehalose or (non adj reducing adj saccharide$1)) near6 (synthes$8 or produc$8 or form$6) near6 enzym$8) or ((trehalose or (non adj reducing adj saccharide$1)) near6 (synthes$8 or produc$8 or FORM$6(FORMULAA)) near6 enzym$8) or ((trehalose or (non adj reducing adj saccharide$1)) near6 (synthes$8 or PRODUC$8(PRODUCT-11.1) or FORM$6(FORMULAA)) near6 enzym$8)	USPAT; US-PGPUB	2001/11/09 16:27

L Number	Hits	Search Text	DB	Time stamp
1	299	sulfolobus or acidocaldarius	USPAT; US-PGPUB	2001/11/09 16:07
2	3346	trehalose	USPAT; US-PGPUB	2001/11/09 16:07
3	29	(sulfolobus or acidocaldarius) and trehalose	USPAT; US-PGPUB	2001/11/09 16:07

PGPUB-DOCUMENT-NUMBER: 20010024793
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20010024793 A1

TITLE: Nucleic acid-free thermostable enzymes and methods of production thereof

PUBLICATION-DATE: September 27, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Goldstein, Adam S.	New Market	MD	US	
Hughes, A. John JR.	Germantown	MD	US	

US-CL-CURRENT: 435/6,435/183 ,435/194 ,435/91.1 ,435/91.2

ABSTRACT:

The present invention provides thermostable enzymes, such as DNA polymerases and restriction endonucleases, that are substantially free from contamination with nucleic acids. The invention also provides methods for the production of these enzymes, and kits comprising these enzymes which may be used in amplifying or sequencing nucleic acid molecules, including through use of the polymerase chain reaction (PCR).

DATE FILED: May 30, 2001

BSTX:
[0006] These disruption approaches have several advantages, including their ability to rapidly and completely (in the case of physical methods) disrupt the
bacterial cell such that the release of intracellular proteins is maximized. In fact, these approaches have been used in the initial steps of processes for the purification of a variety of bacterial cytosolic enzymes, including natural
and recombinant proteins from mesophilic organisms such as Escherichia coli, Bacillus subtilis and Staphylococcus aureus (Laurent, S. J., and Vannier, F. S., Biochimie 59:747-750 (1977); Cull, M., and McHenry, C. S., Meth. Enzymol. 182:147-153 (1990); Hughes, A. J., Jr., et al., J. Cell. Biochem. Suppl. 0 16 (Part B):84 (1992); Ausubel, F. M., et al., in: Current Protocols in Molecular Biology, New York: John Wiley & Sons, pp. 4.4.1-4.4.7 (1993)), as well as phosphatases, restriction enzymes, DNA or RNA polymerases and other proteins from thermophilic bacteria and archaea such as Thermus aquaticus, Thermus thermophilus, Thermus flavis, Thermus caldophilus, Thermotoga maritima,
and **Sulfolobus acidocaldarius** (Shinomiya, T., et al., J. Biochem. 92(6):1823-1832 (1982); Elie, C., et al., Biochim. Biophys. Acta 951(2-3):261-267 (1988); Palm, P., et al., Nucl. Acids Res. 21(21):4904-4908 (1993); Park, J. H., et al., Eur. J. Biochem. 214(I):135-140 (1993); Harrell,
R. A., and Hand, R. P., PCR Meth. Appl. 3(6):372-375 (1994); Meyer, W., et al., Arch. Biochem. Biophys. 319(I):149-156 (1995)).

DETX:
[0021] Thermostable enzymes (e.g., DNA polymerases or restriction enzymes) may

be prepared according to the methods of the present invention from a variety of thermophilic bacteria that are available commercially (for example, from American Type Culture Collection, Rockville, Md.). Suitable for use as sources of thermostable enzymes are the thermophilic bacteria Thermus aquaticus, Thermus thermophilus, Thermococcus litoralis, Pyrococcus furiosus, Pyrococcus woosii and other species of the Pyrococcus genus, Bacillus stearothermophilus, **Sulfolobus acidocaldarius**, Thermoplasma acidophilum, Thermus flavus, Thermus ruber, Thermus brockianus, Thermotoga neapolitana, Thermotoga maritima and other species of the Thermotoga genus, and Methanobacterium thermoautotrophicum, and mutants of each of these species. It will be understood by one of ordinary skill in the art, however, that any thermophilic microorganism may be used as a source for preparation of thermostable enzymes according to the methods of the present invention. Bacterial cells may be grown according to standard microbiological techniques, using culture media and incubation conditions suitable for growing active cultures of the particular species that are well-known to one of ordinary skill in the art (see, e.g., Brock, T. D., and Freeze, H., J. Bacteriol. 98(I):289-297 (1969); Oshima, T., and Imahori, K, Int. J. Syst. Bacteriol. 24(I):102-112 (1974)).

DETX:
[0037] Following their purification, the substantially DNA-free thermostable enzymes may be stored until use in a buffered solution at temperatures of about -80.degree. to 25.degree. C., most preferably at -80.degree. to 4.degree. C., or in lyophilized form. Alternatively, the enzymes may be stabilized by drying in the presence of a sugar such as **trehalose** (U.S. Pat. Nos. 5,098,893 and 4,824,938) or acacia gum, pectin, carboxymethylcellulose, carboxymethylhydroxyethylcellulose, guar, carboxy guar, carboxymethylhydroxypropyl guar, laminaran, chitin, alginates or carrageenan. In addition, the enzymes provided by the present invention may be directly formulated into compositions to be used in techniques requiring the use of thermostable enzymes, such as compositions for nucleic acid sequencing or amplification in the case of thermostable DNA polymerases such as Taq, Tne, or Tma DNA polymerases, or mutants, derivatives or fragments thereof. These formulations may be concentrated solutions of the enzymes, or solutions of the enzymes at working concentrations which may comprise additional components and which may be prepared as described in co-pending U.S. patent application Ser. No. 08/689,815, by Ayoub Rashtchian and Joseph Solus, entitled "Stable Compositions for Nucleic Acid Sequencing and Amplification," filed Aug. 14, 1996, which is incorporated by reference herein in its entirety.

US-CL-CURRENT: 127/40,127/55 ,435/105 ,435/98 ,435/99

US-PAT-NO: 6303346
DOCUMENT-IDENTIFIER: US 6303346 B1
TITLE: Method of producing saccharide preparations
DATE-ISSUED: October 16, 2001
INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Liaw; Gin C.	Decatur	IL	N/A	N/A
Pedersen; Sven	Gentofte	N/A	N/A	DKX
Hendriksen; Hanne Vang	Holte	N/A	N/A	DKX
Svendsen; Allan	Birker.o	N/A	N/A	DKX
Nielsen; Bjarne R.o	slashed.d	N/A	N/A	DKX
slashed.nfeldt	Virum	N/A	N/A	DKX
Nielsen; Ruby Illum	Farum			

US-CL-CURRENT: 435/96,127/40 ,127/55 ,435/105 ,435/98 ,435/99
ABSTRACT:
The present invention relates to a method for the production of saccharide preparations, i.e., syrups, by saccharifying a liquefied starch solution, which
method comprises a saccharification step during which step one or more enzymatic saccharification stages takes place, and the subsequent steps of one or more high temperature membrane separation steps, and re-circulation of the saccharification enzyme, in which method the membrane separation steps are carried out as an integral part of the saccharification step. In another specific aspect, the invention provides a method of producing a saccharide preparation, which method comprises an enzymatic saccharification step, and the
subsequent steps of one or more high temperature membrane separation steps and re-circulation of the saccharification enzyme.
10 Claims, 5 Drawing figures
Exemplary Claim Number: 1,5,8
Number of Drawing Sheets: 5

BSPR:
The present invention relates to the production of mono and/or oligosaccharides
from starch, including dextrose, **trehalose,** isomaltooligosaccaharides, cyclodextrins and maltooligosaccharides. In a specific aspect, the invention provides a method of saccharifying a liquefied starch solution, which method comprises a saccharification step during which step one or more enzymatic saccharification stages takes place, and the subsequent steps of one or more high temperature membrane separation steps, and re-circulation of the saccharification enzyme, in which method the membrane separation steps are carried out as an integral part of the saccharification step.

BSPR:
In another specific aspect, the invention provides a method of producing a mono
and/or oligosaccharide, such as dextrose, **trehalose,** isomaltooligosaccaharide, cyclodextrins and maltooligosaccharide preparation, which method comprises an enzymatic saccharification step, and the subsequent steps of one or more high temperature membrane separation steps and re-circulation of the saccharification enzyme.

BSPR:

Trehalose (.alpha.-D-glucopyranosyl .alpha.-D-glucopyranoside) is a non reducing disaccharide with two glucose residues bound by a .alpha.-1,1 linkage.

BSPR:
Enzymatic processes for producing **trehalose** from starch or maltooligosaccharides are described by, e.g., Kato et al., (1996), Biosci. Biotech. Biochem., 60 (3), p. 546-550); Kazuhisa et al. (1997), Starch 49, no.
1. p. 26-30; and in EP 764,720.

BSPR:
It has now been found that in a method of producing mono and/or oligosaccharides from starch, including dextrose, **trehalose,** isomaltooligosaccharides, cyclodextrins and maltooligosaccharides, the efficiency can be improved significantly, and the costs lowered, if in the saccharification (or hydrolyzing) step, after the liquefaction step, the syrup is subjected to one or more high temperature membrane separation steps, and the
saccharification enzyme is returned to the saccharification step. According to
the method of the present invention, the membrane separation step may be regarded as an integral part of the saccharification step.

BSPR:
When producing saccharides with more than one saccharide unit, i.e., **trehalose,**
isomaltooligosaccharides, cyclodextrins and maltooligosaccharides the hydrolyzing step (after the liquefaction step) is followed by an ultra and microfiltration step or a micro and ultrafiltration step.

BSPR:
In its second aspect, the invention provides a method for the production of a mono and/or oligosaccharide preparation of, e.g., dextrose, **trehalose,** isomaltooligosaccharides, cyclodextrins and maltooligosaccharides, which method
comprises an enzymatic saccharification step, and the subsequent steps of

BSPL:
Trehalose Syrups

DEPR:
According to the method of the invention, the retentate from the membrane separation is conveyed back (re-circulated) to the saccharification step. Preferably the retentate from the membrane separation is re-circulated to a saccharification stage in the saccharification step, at which stage the content
of the reaction mixture matches the content of the retentate with respect to the saccharide, such as glucose, **trehalose,** isomaltooligosaccharide, cyclodextrin or maltooligosaccharide.

DEPR:
When producing **trehalose,** the feed. stream subjected to membrane separation originating from the saccharification stage holds of from about 50 to about 90%, preferably of from about 60 to 90%, more preferred of from about 75-90% **trehalose**.

DEPR:
In the context of the present invention a membrane separation step comprises a

microfiltration step followed by an ultrafiltration step or an ultrafiltration step followed by a microfiltration step when producing **trehalose,** isomaltooligosaccharides, cyclodextrins and maltooligosaccharides.

DEPR:
In a preferred embodiment, the membrane separation steps comprises a microfiltration step and an ultrafiltration step, applied in the order specified. This embodiment is particularly useful for the production of a syrup holding from about 95 to about 96% glucose, or from 10-40% isomaltose, or
30 to above 80% maltose, or 75-90% **trehalose,** or 30-60% cyclodextrins.

DEPR:
In the saccharification step, when producing **trehalose,** liquefied starch is subjected to the action of an enzyme capable of first converting maltooligosaccharide (from the liquefaction step) into the non reducing saccharide maltooligosyl **trehalose** by intramolecular transglycosylation followed by a subsequent step of hydrolyzing the reaction product of the first step (i.e., maltooligosyl **trehalose) into trehalose**. The saccharification step
may be performed using maltooligosyl **trehalose** synthase (MTSase) and maltooligosyl **trehalose** trehalohydrolase (MTHase), e.g., the two enzymes described by Masaru et al. (1996), Biosci. Biotech. Biochem., 60 (3), 546-550). MTSase and MTHase act on amylose or starch to produce **trehalose**.

DEPR:
Another enzymatic process for producing **trehalose** from starch or maltooligosaccharides (see Kato et al., (1996), Biosci. Biotech. Biochem., 60
(3), p. 546-550) involves using **trehalose**-producing enzymes, a glycosyltransferase and an amylase, respectively, from the hyperthermophilic archae **Sulfolobus** solfataricus KM1.

DEPR:
Further, EP 764720 also describes using two enzymes from Solfolobus spp. for preparing **trehalose** from starch or maltooligosacchairdes.

DEPR:
When producing **trehalose,** the feed stream subjected to membrane separation originating from the saccharification stage holds of from about 50 to about 90%, preferably of from about 60 to 90%, more preferred of from about 75-90% **trehalose**.

DEPR:
A thermostable isoamylase may be derived from a strain of Flavobacterium, in particular Flavobacterium odoratum, a strain derived from the thermophilic acrhaebacterium **Sulfolobus acidocaldarius** (Hayashibara, (1996) Biochimica et Biophysica Acta 1291, p. 177-181, such as **Sulfolobus acidocaldarius** ATCC33909 and from a strain of Rhodethermus marius.

DEPR:
Preferably, the saccharification step, when producing **trehalose** is performed in
presence of a MTSase and MTHase, e.g., the enzymes disclosed by Masaru et al. (1996), Biosci. Biotech. Biochem., 60 (3), 546-550).

DEPL:
Production of **Trehalose** (containing 75-90% **trehalose**)

US-PAT-NO: 6284534
DOCUMENT-IDENTIFIER: US 6284534 B1
TITLE: Yeast vector comprising a shortened promoter sequence
DATE-ISSUED: September 4, 2001
INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Kondo; Keiji	Yokohama	N/A	N/A	JPX
Miura; Yutaka	Yokohama	N/A	N/A	JPX

US-CL-CURRENT: 435/320.1
ABSTRACT:

An object of the present invention is to provide a vector which can be integrated into a yeast chromosome in a high number of copies. Another object of the present invention is to provide a modified vector which can be integrated into the yeast chromosome in a high number of copies and of which expression units stably maintain on the chromosome. The vector according to the present invention comprises a marker gene for selecting transformants, a shortened promoter sequence which is operably linked to the marker gene and a sequence homologous to the chromosomal DNA of Candida utilis, and optionally a heterologous gene or a gene derived from C. utilis, wherein the vector is linearized by cleaving within said homologous DNA sequence or at both ends of the homologous DNA sequence with restriction enzymes, and wherein the heterologous gene or the gene derived from C. utilis can be integrated into the chromosomal DNA of C. utilis by homologous recombination.

16 Claims, 29 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 29

DEPR:
The amino acid sequence encoded by the amylase gene derived from **Sulfolobus** solfataricus KM1 (Kobayashi K. et al., Biosci. Biotech. Biochem., 60(10), 1720-1723, 1966), was converted to the DNA sequence using those codons, except those for methionine and tryptophane, most frequently used in the glyceraldehyde-3-phosphoric acid dehydrogenase (GAP) gene of C. utilis. The DNA sequence was designed such that the variation in codons for each amino acid contained in the gene would be as close as possible to that for GAP, that specific restriction enzyme sites would be formed at intervals of about 180 to 320 bases, and that the gene would be constructed as a group of several segments. Some minor codons were also used to conveniently form the restriction enzyme cleavage sites. In addition, the sequence was designed to have an XbaI recognition site on the 5' upstream side one base distant from the translation initiation codon (ATG) of the structural gene and a BglII recognition site on the 3' downstream side one base distant from the translation termination codon. Taking these design parameters into consideration, the gene encoding the amylase from S. solfataricus KM1 was constructed to consist of seven segments, A-1 to A-7 (SEQ. ID. NO. 7 to 13). Each segment has specific restriction enzyme recognition sites at both ends and additional two nonsense nucleotides at both ends of the restriction enzyme recognition sites to enable each segment to be digested directly by the restriction enzymes. Primers used in the synthesis of each segment are shown in FIGS. 24, 25 and 26.

ORPL:
Kobayashi, K., et al., "Gene Analysis of **Trehalose**-Producing Enzymes from

Hypertherophilic Archaea in Sulfolobales," Biosci. Biotech. Biochem., vol. 60, No. 10, 1720-1723 (1996).

US-CL-CURRENT: 435/183,435/194 ,435/91.2 ,530/350

US-PAT-NO: 6245533
DOCUMENT-IDENTIFIER: US 6245533 B1
TITLE: Nucleic acid-free thermostable enzymes and methods of production thereof
DATE-ISSUED: June 12, 2001
INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Goldstein; Adam S.	New Market	MD	N/A	N/A
Hughes, Jr.; A. John	Germantown	MD	N/A	N/A

US-CL-CURRENT: 435/91.1,435/183 ,435/194 ,435/91.2 ,530/350
ABSTRACT:
The present invention provides thermostable enzymes, such as DNA polymerases and restriction endonucleases, that are substantially free from contamination with nucleic acids. The invention also provides methods for the production of these enzymes, and kits comprising these enzymes which may be used in amplifying or sequencing nucleic acid molecules, including through use of the polymerase chain reaction (PCR).
18 Claims, 2 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 2

BSPR:
These disruption approaches have several advantages, including their ability to rapidly and completely (in the case of physical methods) disrupt the bacterial cell such that the release of intracellular proteins is maximized. In fact, these approaches have been used in the initial steps of processes for the purification of a variety of bacterial cytosolic enzymes, including natural and recombinant proteins from mesophilic organisms such as Escherichia coli, Bacillus subtilis and Staphylococcus aureus (Laurent, S. J., and Vannier, F. S., Biochimie 59:747-750 (1977); Cull, M., and McHenry, C. S., Meth. Enzymol. 182:147-153 (1990); Hughes, A. J., Jr., et al., J. Cell. Biochem. Suppl. 0 16(Part B):84 (1992); Ausubel, F. M., et al., in: Current Protocols in Molecular Biology, New York: John Wiley & Sons, pp. 4.4.1-4.4.7 (1993)), as well as phosphatases, restriction enzymes, DNA or RNA polymerases and other proteins from thermophilic bacteria and archaea such as Thermus aquaticus, Thermus thermophilus, Thermus flavis, Thermus caldophilus, Thermotoga maritima, and **Sulfolobus acidocaldarius** (Shinomiya, T., et al., J. Biochem. 92(6):1823-1832 (1982); Elie, C., et al., Biochim. Biophys. Acta 951(2-3):261-267 (1988): Palm, P., et al., Nucl. Acids Res. 21(21):4904-4908 (1993); Park, J. H., et al., Eur. J. Biochem. 214(1):135-140 (1993); Harrell, R. A., and Hand, R.P., PCR Meth. Appl. 3(6):372-375 (1994); Meyer, W., et al., Arch. Biochem. Biophys. 319(1):149-156 (1995)).

DEPR:
Thermostable enzymes (e.g., DNA polymerases or restriction enzymes) may be prepared according to the methods of the present invention from a variety of thermophilic bacteria that are available commercially (for example, from American Type Culture Collection, Rockville, Md.). Suitable for use as sources

of thermostable enzymes are the thermophilic bacteria Thermus aquaticus, Thermus thermophilus, Thermococcus litoralis, Pyrococcus furiosus, Pyrococcus woosii and other species of the Pyrococcus genus, Bacillus stearothermophilus, **Sulfolobus acidocaldarius**, Thermoplasma acidophilum, Thermus flavus, Thermus ruber, Thermus brockianus, Thermotoga neapolitana, Thermotoga maritima and other species of the Thermotoga genus, and Methanobacterium thermoautotrophicum, and mutants of each of these species. It will be understood by one of ordinary skill in the art, however, that any thermophilic microorganism may be used as a source for preparation of thermostable enzymes according to the methods of the present invention. Bacterial cells may be grown according to standard microbiological techniques, using culture media and
incubation conditions suitable for growing active cultures of the particular species that are well-known to one of ordinary skill in the art (see, e.g., Brock, T. D., and Freeze, H., J. Bacteriol. 98(1):289-297 (1969); Oshima, T., and Imahori, K, Int. J. Syst. Bacteriol. 24(1):102-112 (1974)).

DEPR:
Following their purification, the substantially DNA-free thermostable enzymes may be stored until use in a buffered solution at temperatures of about -80.degree. to 25.degree. C., most preferably at -80.degree. to 4.degree. C., or in lyophilized form. Alternatively, the enzymes may be stabilized by drying in the presence of a sugar such as **trehalose** (U.S. Pat. Nos. 5,098,893 and 4,824,938) or acacia gum, pectin, carboxymethylcellulose, carboxymethylhydroxyethylcellulose, guar, carboxy guar, carboxymethylhydroxypropyl guar, laminaran, chitin, alginates or carrageenan. In addition, the enzymes provided by the present invention may be directly formulated into compositions to be used in techniques requiring the use of thermostable enzymes, such as compositions for nucleic acid sequencing or amplification in the case of thermostable DNA polymerases such as Taq, Tne, or Tma DNA polymerases, or mutants, derivatives or fragments thereof These formulations may be concentrated solutions of the enzymes, or solutions of the enzymes at working concentrations which may comprise additional components and which may be prepared as described in co-pending U.S. patent application Ser. No. 08/689,815, by Ayoub Rashtchian and Joseph Solus, entitled "Stable Compositions for Nucleic Acid Sequencing and Amplification," filed Aug. 14, 1996, which is incorporated by reference herein in its entirety.

ORPL:
Meyer, W., et al., "Purification, Cloning, and Sequencing of Archaebacterial Pyrophosphatasr from the Extreme Thermoacidophile **Sulfolobus acidocaldarius**," Arch. Biochem. Biophys. 319:149-156 (1995).

L Number	Hits	Search Text	DB	Time stamp
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-	18	(sulfolobus or acidocaldarius) and (non adj reducing adj saccharide$1)	USPAT; US-PGPUB	2001/11/09 16:12

US-CL-CURRENT: 127/40,127/55 ,435/105 ,435/98 ,435/99

US-PAT-NO: 6303346
DOCUMENT-IDENTIFIER: US 6303346 B1
TITLE: Method of producing saccharide preparations
DATE-ISSUED: October 16, 2001
INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Liaw; Gin C.	Decatur	IL	N/A	N/A
Pedersen; Sven	Gentofte	N/A	N/A	DKX
Hendriksen; Hanne Vang	Holte	N/A	N/A	DKX
Svendsen; Allan	Birker.o	N/A	N/A	DKX
Nielsen; Bjarne R.o	slashed.d	N/A	N/A	DKX
slashed.nfeldt	Virum	N/A	N/A	DKX
Nielsen; Ruby Illum	Farum			

US-CL-CURRENT: 435/96,127/40 ,127/55 ,435/105 ,435/98 ,435/99
ABSTRACT:
The present invention relates to a method for the production of saccharide preparations, i.e., syrups, by saccharifying a liquefied starch solution, which
method comprises a saccharification step during which step one or more enzymatic saccharification stages takes place, and the subsequent steps of one or more high temperature membrane separation steps, and re-circulation of the saccharification enzyme, in which method the membrane separation steps are carried out as an integral part of the saccharification step. In another specific aspect, the invention provides a method of producing a saccharide preparation, which method comprises an enzymatic saccharification step, and the
subsequent steps of one or more high temperature membrane separation steps and re-circulation of the saccharification enzyme.
10 Claims, 5 Drawing figures
Exemplary Claim Number: 1,5,8
Number of Drawing Sheets: 5

DEPR:
In the saccharification step, when producing trehalose, liquefied starch is subjected to the action of an enzyme capable of first converting maltooligosaccharide (from the liquefaction step) into the **non reducing saccharide** maltooligosyl trehalose by intramolecular transglycosylation followed by a subsequent step of hydrolyzing the reaction product of the first step (i.e., maltooligosyl trehalose) into trehalose. The saccharification step
may be performed using maltooligosyl trehalose synthase (MTSase) and maltooligosyl trehalose trehalohydrolase (MTHase), e.g., the two enzymes described by Masaru et al. (1996), Biosci. Biotech. Biochem., 60 (3), 546-550). MTSase and MTHase act on amylose or starch to produce trehalose.

DEPR:
Another enzymatic process for producing trehalose from starch or maltooligosaccharides (see Kato et al., (1996), Biosci. Biotech. Biochem., 60
(3), p. 546-550) involves using trehalose-producing enzymes, a glycosyltransferase and an amylase, respectively, from the hyperthermophilic archae **Sulfolobus** solfataricus KM1.

DEPR:

A thermostable isoamylase may be derived from a strain of Flavobacterium, in particular Flavobacterium odoratum, a strain derived from the thermophilic acrhaebacterium **Sulfolobus acidocaldarius** (Hayashibara, (1996) Biochimica et Biophysica Acta 1291, p. 177-181, such as **Sulfolobus acidocaldarius** ATCC33909 and from a strain of Rhodethermus marius.

L Number	Hits	Search Text	DB	Time stamp
1	299	sulfolobus or acidocaldarius	USPAT; US-PGPUB	2001/11/09 16:07
2	3346	trehalose	USPAT; US-PGPUB	2001/11/09 16:07
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4	98	non adj reducing adj saccharide$1	USPAT; US-PGPUB	2001/11/09 16:11
5	18	(sulfolobus or acidocaldarius) and (non adj reducing adj saccharide$1)	USPAT; US-PGPUB	2001/11/09 16:12
6	3366	$trehalose	USPAT; US-PGPUB	2001/11/09 16:12
7	29	(sulfolobus or acidocaldarius) and $trehalose	USPAT; US-PGPUB	2001/11/09 16:12

PGPUB-DOCUMENT-NUMBER: 20010024793
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20010024793 A1

TITLE: Nucleic acid-free thermostable enzymes and methods of production thereof

PUBLICATION-DATE: September 27, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Goldstein, Adam S.	New Market	MD	US	
Hughes, A. John JR.	Germantown	MD	US	

US-CL-CURRENT: 435/6,435/183 ,435/194 ,435/91.1 ,435/91.2

ABSTRACT:

The present invention provides thermostable enzymes, such as DNA polymerases and restriction endonucleases, that are substantially free from contamination with nucleic acids. The invention also provides methods for the production of these enzymes, and kits comprising these enzymes which may be used in amplifying or sequencing nucleic acid molecules, including through use of the polymerase chain reaction (PCR).

DATE FILED: May 30, 2001

BSTX:
[0006] These disruption approaches have several advantages, including their ability to rapidly and completely (in the case of physical methods) disrupt the
bacterial cell such that the release of intracellular proteins is maximized. In fact, these approaches have been used in the initial steps of processes for the purification of a variety of bacterial cytosolic enzymes, including natural
and recombinant proteins from mesophilic organisms such as Escherichia coli, Bacillus subtilis and Staphylococcus aureus (Laurent, S. J., and Vannier, F. S., Biochimie 59:747-750 (1977); Cull, M., and McHenry, C. S., Meth. Enzymol. 182:147-153 (1990); Hughes, A. J., Jr., et al., J. Cell. Biochem. Suppl. 0 16 (Part B):84 (1992); Ausubel, F. M., et al., in: Current Protocols in Molecular Biology, New York: John Wiley & Sons, pp. 4.4.1-4.4.7 (1993)), as well as phosphatases, restriction enzymes, DNA or RNA polymerases and other proteins from thermophilic bacteria and archaea such as Thermus aquaticus, Thermus thermophilus, Thermus flavis, Thermus caldophilus, Thermotoga maritima,
and **Sulfolobus acidocaldarius** (Shinomiya, T., et al., J. Biochem. 92(6):1823-1832 (1982); Elie, C., et al., Biochim. Biophys. Acta 951(2-3):261-267 (1988); Palm, P., et al., Nucl. Acids Res. 21(21):4904-4908 (1993); Park, J. H., et al., Eur. J. Biochem. 214(I):135-140 (1993); Harrell,
R. A., and Hand, R. P., PCR Meth. Appl. 3(6):372-375 (1994); Meyer, W., et al., Arch. Biochem. Biophys. 319(I):149-156 (1995)).

DETX:
[0021] Thermostable enzymes (e.g., DNA polymerases or restriction enzymes) may

be prepared according to the methods of the present invention from a variety of thermophilic bacteria that are available commercially (for example, from American Type Culture Collection, Rockville, Md.). Suitable for use as sources of thermostable enzymes are the thermophilic bacteria Thermus aquaticus, Thermus thermophilus, Thermococcus litoralis, Pyrococcus furiosus, Pyrococcus woosii and other species of the Pyrococcus genus, Bacillus stearothermophilus, **Sulfolobus acidocaldarius**, Thermoplasma acidophilum, Thermus flavus, Thermus ruber, Thermus brockianus, Thermotoga neapolitana, Thermotoga maritima and other species of the Thermotoga genus, and Methanobacterium thermoautotrophicum, and mutants of each of these species. It will be understood by one of ordinary skill in the art, however, that any thermophilic microorganism may be used as a source for preparation of thermostable enzymes according to the methods of the present invention. Bacterial cells may be grown according to standard microbiological techniques, using culture media and incubation conditions suitable for growing active cultures of the particular species that are well-known to one of ordinary skill in the art (see, e.g., Brock, T. D., and Freeze, H., J. Bacteriol. 98(I):289-297 (1969); Oshima, T., and Imahori, K, Int. J. Syst. Bacteriol. 24(I):102-112 (1974)).

DETX:
[0037] Following their purification, the substantially DNA-free thermostable enzymes may be stored until use in a buffered solution at temperatures of about -80.degree. to 25.degree. C., most preferably at -80.degree. to 4.degree. C., or in lyophilized form. Alternatively, the enzymes may be stabilized by drying in the presence of a sugar such as **trehalose** (U.S. Pat. Nos. 5,098,893 and 4,824,938), or acacia gum, pectin, carboxymethylcellulose, carboxymethylhydroxyethylcellulose, guar, carboxy guar, carboxymethylhydroxypropyl guar, laminaran, chitin, alginates or carrageenan. In addition, the enzymes provided by the present invention may be directly formulated into compositions to be used in techniques requiring the use of thermostable enzymes, such as compositions for nucleic acid sequencing or amplification in the case of thermostable DNA polymerases such as Taq, Tne, or Tma DNA polymerases, or mutants, derivatives or fragments thereof. These formulations may be concentrated solutions of the enzymes, or solutions of the enzymes at working concentrations which may comprise additional components and which may be prepared as described in co-pending U.S. patent application Ser. No. 08/689,815, by Ayoub Rashtchian and Joseph Solus, entitled "Stable Compositions for Nucleic Acid Sequencing and Amplification," filed Aug. 14, 1996, which is incorporated by reference herein in its entirety.

US-CL-CURRENT: 127/40,127/55 ,435/105 ,435/98 ,435/99

US-PAT-NO: 6303346
DOCUMENT-IDENTIFIER: US 6303346 B1
TITLE: Method of producing saccharide preparations
DATE-ISSUED: October 16, 2001
INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Liaw; Gin C.	Decatur	IL	N/A	N/A
Pedersen; Sven	Gentofte	N/A	N/A	DKX
Hendriksen; Hanne Vang	Holte	N/A	N/A	DKX
Svendsen; Allan	Birker.o	N/A	N/A	DKX
Nielsen; Bjarne R.o	slashed.d	N/A	N/A	DKX
slashed.nfeldt	Virum	N/A	N/A	DKX
Nielsen; Ruby Illum	Farum			

US-CL-CURRENT: 435/96,127/40 ,127/55 ,435/105 ,435/98 ,435/99
ABSTRACT:
The present invention relates to a method for the production of saccharide preparations, i.e., syrups, by saccharifying a liquefied starch solution, which
method comprises a saccharification step during which step one or more enzymatic saccharification stages takes place, and the subsequent steps of one or more high temperature membrane separation steps, and re-circulation of the saccharification enzyme, in which method the membrane separation steps are carried out as an integral part of the saccharification step. In another specific aspect, the invention provides a method of producing a saccharide preparation, which method comprises an enzymatic saccharification step, and the
subsequent steps of one or more high temperature membrane separation steps and re-circulation of the saccharification enzyme.
10 Claims, 5 Drawing figures
Exemplary Claim Number: 1,5,8
Number of Drawing Sheets: 5

BSPR:
The present invention relates to the production of mono and/or oligosaccharides
from starch, including dextrose, **trehalose**, isomaltooligosaccaharides, cyclodextrins and maltooligosaccharides. In a specific aspect, the invention provides a method of saccharifying a liquefied starch solution, which method comprises a saccharification step during which step one or more enzymatic saccharification stages takes place, and the subsequent steps of one or more high temperature membrane separation steps, and re-circulation of the saccharification enzyme, in which method the membrane separation steps are carried out as an integral part of the saccharification step.

BSPR:
In another specific aspect, the invention provides a method of producing a mono
and/or oligosaccharide, such as dextrose, **trehalose**, isomaltooligosaccaharide, cyclodextrins and maltooligosaccharide preparation, which method comprises an enzymatic saccharification step, and the subsequent steps of one or more high temperature membrane separation steps and re-circulation of the saccharification enzyme.

BSPR:

Trehalose (.alpha.-D-glucopyranosyl .alpha.-D-glucopyranoside) is a non reducing disaccharide with two glucose residues bound by a .alpha.-1,1 linkage.

BSPR:
Enzymatic processes for producing **trehalose** from starch or maltooligosaccharides are described by, e.g., Kato et al., (1996), Biosci. Biotech. Biochem., 60 (3), p. 546-550); Kazuhisa et al. (1997), Starch 49, no.
1. p. 26-30; and in EP 764,720.

BSPR:
It has now been found that in a method of producing mono and/or oligosaccharides from starch, including dextrose, **trehalose,** isomaltooligosaccharides, cyclodextrins and maltooligosaccharides, the efficiency can be improved significantly, and the costs lowered, if in the saccharification (or hydrolyzing) step, after the liquefaction step, the syrup is subjected to one or more high temperature membrane separation steps, and the
saccharification enzyme is returned to the saccharification step. According to
the method of the present invention, the membrane separation step may be regarded as an integral part of the saccharification step.

BSPR:
When producing saccharides with more than one saccharide unit, i.e., **trehalose,**
isomaltooligosaccharides, cyclodextrins and maltooligosaccharides the hydrolyzing step (after the liquefaction step) is followed by an ultra and microfiltration step or a micro and ultrafiltration step.

BSPR:
In its second aspect, the invention provides a method for the production of a mono and/or oligosaccharide preparation of, e.g., dextrose, **trehalose,** isomaltooligosaccharides, cyclodextrins and maltooligosaccharides, which method
comprises an enzymatic saccharification step, and the subsequent steps of

BSPL:
Trehalose Syrups

DEPR:
According to the method of the invention, the retentate from the membrane separation is conveyed back (re-circulated) to the saccharification step. Preferably the retentate from the membrane separation is re-circulated to a saccharification stage in the saccharification step, at which stage the content
of the reaction mixture matches the content of the retentate with respect to the saccharide, such as glucose, **trehalose,** isomaltooligosaccharide, cyclodextrin or maltooligosaccharide.

DEPR:
When producing **trehalose,** the feed. stream subjected to membrane separation originating from the saccharification stage holds of from about 50 to about 90%, preferably of from about 60 to 90%, more preferred of from about 75-90% **trehalose**.

DEPR:
In the context of the present invention a membrane separation step comprises a

microfiltration step followed by an ultrafiltration step or an ultrafiltration step followed by a microfiltration step when producing **trehalose,** isomaltooligosaccharides, cyclodextrins and maltooligosaccharides.

DEPR:
In a preferred embodiment, the membrane separation steps comprises a microfiltration step and an ultrafiltration step, applied in the order specified. This embodiment is particularly useful for the production of a syrup holding from about 95 to about 96% glucose, or from 10-40% isomaltose, or
30 to above 80% maltose, or 75-90% **trehalose,** or 30-60% cyclodextrins.

DEPR:
In the saccharification step, when producing **trehalose,** liquefied starch is subjected to the action of an enzyme capable of first converting maltooligosaccharide (from the liquefaction step) into the non reducing saccharide maltooligosyl **trehalose** by intramolecular transglycosylation followed by a subsequent step of hydrolyzing the reaction product of the first step (i.e., maltooligosyl **trehalose) into trehalose**. The saccharification step
may be performed using maltooligosyl **trehalose** synthase (MTSase) and maltooligosyl **trehalose** trehalohydrolase (MTHase), e.g., the two enzymes described by Masaru et al. (1996), Biosci. Biotech. Biochem., 60 (3), 546-550). MTSase and MTHase act on amylose or starch to produce **trehalose**.

DEPR:
Another enzymatic process for producing **trehalose** from starch or maltooligosaccharides (see Kato et al., (1996), Biosci. Biotech. Biochem., 60
(3), p. 546-550) involves using **trehalose**-producing enzymes, a glycosyltransferase and an amylase, respectively, from the hyperthermophilic archae **Sulfolobus** solfataricus KM1.

DEPR:
Further, EP 764720 also describes using two enzymes from Solfolobus spp. for preparing **trehalose** from starch or maltooligosacchairdes.

DEPR:
When producing **trehalose,** the feed stream subjected to membrane separation originating from the saccharification stage holds of from about 50 to about 90%, preferably of from about 60 to 90%, more preferred of from about 75-90% **trehalose**.

DEPR:
A thermostable isoamylase may be derived from a strain of Flavobacterium, in particular Flavobacterium odoratum, a strain derived from the thermophilic acrhaebacterium **Sulfolobus acidocaldarius** (Hayashibara, (1996) Biochimica et Biophysica Acta 1291, p. 177-181, such as **Sulfolobus acidocaldarius** ATCC33909 and from a strain of Rhodethermus marius.

DEPR:
Preferably, the saccharification step, when producing **trehalose** is performed in
presence of a MTSase and MTHase, e.g., the enzymes disclosed by Masaru et al. (1996), Biosci. Biotech. Biochem., 60 (3), 546-550).

DEPL:
Production of **Trehalose** (containing 75-90% **trehalose**)

US-PAT-NO: 6284534
DOCUMENT-IDENTIFIER: US 6284534 B1
TITLE: Yeast vector comprising a shortened promoter sequence
DATE-ISSUED: September 4, 2001
INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Kondo; Keiji	Yokohama	N/A	N/A	JPX
Miura; Yutaka	Yokohama	N/A	N/A	JPX

US-CL-CURRENT: 435/320.1
ABSTRACT:

An object of the present invention is to provide a vector which can be integrated into a yeast chromosome in a high number of copies. Another object of the present invention is to provide a modified vector which can be integrated into the yeast chromosome in a high number of copies and of which expression units stably maintain on the chromosome. The vector according to the present invention comprises a marker gene for selecting transformants, a shortened promoter sequence which is operably linked to the marker gene and a sequence homologous to the chromosomal DNA of Candida utilis, and optionally a heterologous gene or a gene derived from C. utilis, wherein the vector is linearized by cleaving within said homologous DNA sequence or at both ends of the homologous DNA sequence with restriction enzymes, and wherein the heterologous gene or the gene derived from C. utilis can be integrated into the chromosomal DNA of C. utilis by homologous recombination.

16 Claims, 29 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 29

DEPR:
The amino acid sequence encoded by the amylase gene derived from **Sulfolobus** solfataricus KM1 (Kobayashi K. et al., Biosci. Biotech. Biochem., 60(10), 1720-1723, 1966), was converted to the DNA sequence using those codons, except those for methionine and tryptophane, most frequently used in the glyceraldehyde-3-phosphoric acid dehydrogenase (GAP) gene of C. utilis. The DNA sequence was designed such that the variation in codons for each amino acid contained in the gene would be as close as possible to that for GAP, that specific restriction enzyme sites would be formed at intervals of about 180 to 320 bases, and that the gene would be constructed as a group of several segments. Some minor codons were also used to conveniently form the restriction enzyme cleavage sites. In addition, the sequence was designed to have an XbaI recognition site on the 5' upstream side one base distant from the translation initiation codon (ATG) of the structural gene and a BglII recognition site on the 3' downstream side one base distant from the translation termination codon. Taking these design parameters into consideration, the gene encoding the amylase from S. solfataricus KM1 was constructed to consist of seven segments, A-1 to A-7 (SEQ. ID. NO. 7 to 13). Each segment has specific restriction enzyme recognition sites at both ends and additional two nonsense nucleotides at both ends of the restriction enzyme recognition sites to enable each segment to be digested directly by the restriction enzymes. Primers used in the synthesis of each segment are shown in FIGS. 24, 25 and 26.

ORPL:
Kobayashi, K., et al., "Gene Analysis of **Trehalose**-Producing Enzymes from

Hypertherophilic Archaea in Sulfolobales," Biosci. Biotech. Biochem., vol. 60, No. 10, 1720-1723 (1996).

US-CL-CURRENT: 435/183,435/194 ,435/91.2 ,530/350

US-PAT-NO: 6245533
DOCUMENT-IDENTIFIER: US 6245533 B1
TITLE: Nucleic acid-free thermostable enzymes and methods of production thereof
DATE-ISSUED: June 12, 2001
INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Goldstein; Adam S.	New Market	MD	N/A	N/A
Hughes, Jr.; A. John	Germantown	MD	N/A	N/A

US-CL-CURRENT: 435/91.1,435/183 ,435/194 ,435/91.2 ,530/350
ABSTRACT:
The present invention provides thermostable enzymes, such as DNA polymerases and restriction endonucleases, that are substantially free from contamination with nucleic acids. The invention also provides methods for the production of these enzymes, and kits comprising these enzymes which may be used in amplifying or sequencing nucleic acid molecules, including through use of the polymerase chain reaction (PCR).
18 Claims, 2 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 2

BSPR:
These disruption approaches have several advantages, including their ability to rapidly and completely (in the case of physical methods) disrupt the bacterial cell such that the release of intracellular proteins is maximized. In fact, these approaches have been used in the initial steps of processes for the purification of a variety of bacterial cytosolic enzymes, including natural and recombinant proteins from mesophilic organisms such as Escherichia coli, Bacillus subtilis and Staphylococcus aureus (Laurent, S. J., and Vannier, F. S., Biochimie 59:747-750 (1977); Cull, M., and McHenry, C. S., Meth. Enzymol. 182:147-153 (1990); Hughes, A. J., Jr., et al., J. Cell. Biochem. Suppl. 0 16(Part B):84 (1992); Ausubel, F. M., et al., in: Current Protocols in Molecular Biology, New York: John Wiley & Sons, pp. 4.4.1-4.4.7 (1993)), as well as phosphatases, restriction enzymes, DNA or RNA polymerases and other proteins from thermophilic bacteria and archaea such as Thermus aquaticus, Thermus thermophilus, Thermus flavis, Thermus caldophilus, Thermotoga maritima, and **Sulfolobus acidocaldarius** (Shinomiya, T., et al., J. Biochem. 92(6):1823-1832 (1982); Elie, C., et al., Biochim. Biophys. Acta 951(2-3):261-267 (1988): Palm, P., et al., Nucl. Acids Res. 21(21):4904-4908 (1993); Park, J. H., et al., Eur. J. Biochem. 214(1):135-140 (1993); Harrell, R. A., and Hand, R.P., PCR Meth. Appl. 3(6):372-375 (1994); Meyer, W., et al., Arch. Biochem. Biophys. 319(1):149-156 (1995)).

DEPR:
Thermostable enzymes (e.g., DNA polymerases or restriction enzymes) may be prepared according to the methods of the present invention from a variety of thermophilic bacteria that are available commercially (for example, from American Type Culture Collection, Rockville, Md.). Suitable for use as sources

of thermostable enzymes are the thermophilic bacteria Thermus aquaticus, Thermus thermophilus, Thermococcus litoralis, Pyrococcus furiosus, Pyrococcus woosii and other species of the Pyrococcus genus, Bacillus stearothermophilus, **Sulfolobus acidocaldarius,** Thermoplasma acidophilum, Thermus flavus, Thermus ruber, Thermus brockianus, Thermotoga neapolitana, Thermotoga maritima and other species of the Thermotoga genus, and Methanobacterium thermoautotrophicum, and mutants of each of these species. It will be understood by one of ordinary skill in the art, however, that any thermophilic microorganism may be used as a source for preparation of thermostable enzymes according to the methods of the present invention. Bacterial cells may be grown according to standard microbiological techniques, using culture media and
incubation conditions suitable for growing active cultures of the particular species that are well-known to one of ordinary skill in the art (see, e.g., Brock, T. D., and Freeze, H., J. Bacteriol. 98(1):289-297 (1969); Oshima, T., and Imahori, K, Int. J. Syst. Bacteriol. 24(1):102-112 (1974)).

DEPR:
Following their purification, the substantially DNA-free thermostable enzymes may be stored until use in a buffered solution at temperatures of about -80.degree. to 25.degree. C., most preferably at -80.degree. to 4.degree. C., or in lyophilized form. Alternatively, the enzymes may be stabilized by drying in the presence of a sugar such as **trehalose** (U.S. Pat. Nos. 5,098,893 and 4,824,938) or acacia gum, pectin, carboxymethylcellulose, carboxymethylhydroxyethylcellulose, guar, carboxy guar, carboxymethylhydroxypropyl guar, laminaran, chitin, alginates or carrageenan. In addition, the enzymes provided by the present invention may be directly formulated into compositions to be used in techniques requiring the use of thermostable enzymes, such as compositions for nucleic acid sequencing or amplification in the case of thermostable DNA polymerases such as Taq, Tne, or Tma DNA polymerases, or mutants, derivatives or fragments thereof These formulations may be concentrated solutions of the enzymes, or solutions of the enzymes at working concentrations which may comprise additional components and which may be prepared as described in co-pending U.S. patent application Ser. No. 08/689,815, by Ayoub Rashtchian and Joseph Solus, entitled "Stable Compositions for Nucleic Acid Sequencing and Amplification," filed Aug. 14, 1996, which is incorporated by reference herein in its entirety.

ORPL:
Meyer, W., et al., "Purification, Cloning, and Sequencing of Archaebacterial Pyrophosphatasr from the Extreme Thermoacidophile **Sulfolobus acidocaldarius,**" Arch. Biochem. Biophys. 319:149-156 (1995).

L Number	Hits	Search Text	DB	Time stamp
-	299	sulfolobus or acidocaldarius	USPAT; US-PGPUB	2001/11/09 16:07
-	3346	trehalose	USPAT; US-PGPUB	2001/11/09 16:07
-	29	(sulfolobus or acidocaldarius) and trehalose	USPAT; US-PGPUB	2001/11/09 16:11
-	98	non adj reducing adj saccharide$1	USPAT; US-PGPUB	2001/11/09 16:11
-	18	(sulfolobus or acidocaldarius) and (non adj reducing adj saccharide$1)	USPAT; US-PGPUB	2001/11/09 16:12
-	3366	$trehalose	USPAT; US-PGPUB	2001/11/09 16:12
-	29	(sulfolobus or acidocaldarius) and $trehalose	USPAT; US-PGPUB	2001/11/09 16:12
-	102	(trehalose or (non adj reducing adj saccharide$1)) near6 (synthes$8 or produc$8 or form$6) near6 enzym$8	USPAT; US-PGPUB	2001/11/09 16:19
-	89	(trehalose or (non adj reducing adj saccharide$1)) near6 (synthes$8 or produc$8 or FORM$6(FORMULAA)) near6 enzym$8	USPAT; US-PGPUB	2001/11/09 16:24
-	25	(trehalose or (non adj reducing adj saccharide$1)) near6 (synthes$8 or PRODUC$8(PRODUCT-11.1) or FORM$6(FORMULAA)) near6 enzym$8	USPAT; US-PGPUB	2001/11/09 16:26
-	102	((trehalose or (non adj reducing adj saccharide$1)) near6 (synthes$8 or produc$8 or form$6) near6 enzym$8) or ((trehalose or (non adj reducing adj saccharide$1)) near6 (synthes$8 or produc$8 or FORM$6(FORMULAA)) near6 enzym$8) or ((trehalose or (non adj reducing adj saccharide$1)) near6 (synthes$8 or PRODUC$8(PRODUCT-11.1) or FORM$6(FORMULAA)) near6 enzym$8)	USPAT; US-PGPUB	2001/11/09 16:27

PGPUB-DOCUMENT-NUMBER: 20010033888
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20010033888 A1

TITLE: Crystalline trehalose dihydrate, its preparation and uses

PUBLICATION-DATE: October 25, 2001

US-CL-CURRENT: 426/658,127/58

APPL-NO: 09/846326

DATE FILED: May 2, 2001

RELATED-US-APPL-DATA:
[0001] This is a division of copending parent application Ser. No. 09/349,103,
filed July 8, 1999.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
JP	194356/1998	1998JP-194356/1998	July 9, 1998

BSTX:
[0006] Industrial-scale production of crystalline trehalose dihydrate has been realized, for example, by using in combination several **enzymes, in particular, a non-reducing saccharide-forming enzyme,** as disclosed in Japanese Patent Kokai
No. 143,876/95, which forms a non-reducing saccharide having a trehalose structure as an end unit from one or more reducing partial starch hydrolysates with a glucose polymerization degree of at least three; and a trehalose-releasing enzyme, as disclosed in Japanese Patent Kokai No. 213,283/95, which specifically hydrolyzes the bonding between the part of a trehalose structure and the resting part of a non-reducing saccharide having a trehalose structure as an end unit and a glucose polymerization degree of at least three. Journal of Chemical Physics, Vol. 77, No. 5, pp. 2,330-2,335 (1982) reported the structure of crystalline trehalose dihydrate which has an orthorhombic structure as shown in FIG. 1, and has axes having different lengths and crossings at right angles, i.e., it has the a, b, and c axes, and which more elongates to the direction of the c axis than to the b axis, resulting in growth of an easily fragile slender shape of crystal. In fact, commercially available crystalline trehalose dihydrate has a rather slender shape as shown in FIG. 2, a microscopic photograph, where the proportion of the
length to the direction of the c axis (the length to the direction of the c axis is abbreviated as the c axis throughout the specification, unless specified otherwise) to that of the b axis (the length to the direction of the b axis is abbreviated as the b axis throughout the specification, unless specified otherwise) is about 3.5 to about 5.5 folds and the c axis is about 2 mm at the longest. Commercially available crystalline trehalose dihydrate has a proportion of length to the direction of the c axis to that of the a axis (the length to the direction of the a axis is abbreviated as the a axis throughout the specification, unless specified otherwise) is about 8 to about 12 folds. The aforesaid conventional crystalline trehalose dihydrate is generally too large in surface area, resulting in the following drawbacks: It is not easily separated from molasses in the separation steps; it needs a relatively-large amount of drying energy; and it is easily fragile during the

steps of drying and sieving. Moreover, conventional slender shape of crystalline trehalose dihydrate could not hardly process candy fluff using commercially available machines therefor and hardly be used to process alcoholic beverages with fruits such as ume brandy because the crystal easily dissolves in water. Unlike conventional crystalline trehalose dihydrate, greatly expected is the establishment of a crystalline trehalose dihydrate that
can be easily separated and dried in its processing, free of fracture during the steps of drying and sieving, and arbitrarily used to produce candy fluffs and alcoholic beverages with fruits.

PGPUB-DOCUMENT-NUMBER: 20010031249
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20010031249 A1

TITLE: Composition for inhibiting body odor and uses thereof

PUBLICATION-DATE: October 18, 2001

US-CL-CURRENT: 424/65,424/400 ,424/401 ,442/123 ,514/53

APPL-NO: 09/795522

DATE FILED: March 1, 2001

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
JP	58032/2000	2000JP-58032/2000	March 2, 2000
JP	159204/2000	2000JP-159204/2000	May 29, 2000
JP	202972/2000	2000JP-202972/2000	July 4, 2000
JP	269165/2000	2000JP-269165/2000	September 5, 2000

DETX:
[0012] The trehalose used in the present invention can be prepared by different
methods. With an economical viewpoint, preferable methods are those which comprise a step of contacting with partial starch hydrolysates **non-reducing saccharide-forming enzymes and trehalose**-releasing enzymes as disclosed in Japanese Patent Kokai Nos. 143,876/95, 213,283/95, 322,883/95, 298,880/95, 66,187/96, 66,188/96, 336,388/96, and 84,586/96. These methods can produce trehalose in a satisfactorily-high yield from starches as relatively-low cost materials. Examples of commercially available products prepared by these methods are "TREHALOSE FOR COSMETIC USE", a crystalline trehalose hydrate having a trehalose content of 99% (w/w) or higher, commercialized by Hayashibara Shoji Inc., Okayama, Japan; "TREHA.RTM.", a crystalline trehalose hydrate having a trehalose content of 98% (w/w) or higher, commercialized by Hayashibara Shoji Inc., Okayama, Japan; and "TREHASTAR.RTM.", a trehalose high-content syrup having a trehalose content of 28% (w/w) or higher, commercialized by Hayashibara Shoji Inc., Okayama, Japan. Trehalose can be obtained by contacting maltose either with a maltose/trehalose converting enzyme as disclosed, for example, in either of Japanese Patent Kokai Nos. 170,977/95, 263/96 or 149,980/96; or with a conventionally known maltose phosphorylase or trehalose phosphorylase. Crystalline trehalose anhydride can be prepared by drying crystalline trehalose hydrate similarly as exemplified above under the normal or reduced pressure and at a temperature of 70-160.degree. C., more preferably, under a reduced pressure and at a temperature of 80-100.degree. C.; or by placing in a crystallizer a relatively-high trehalose content solution with a moisture content of less than
10% (w/w), stirring the solution in the presence of seed at a temperature of 50-160.degree. C., preferably, a temperature of 80-140.degree. C. to form a massecuite containing crystalline trehalose anhydride, and crystallizing and pulverizing the resulting massecuite by a method such as block pulverization,

fluidized-bed granulation, or spray-drying. The trehalose products thus obtained can be advantageously used in the present invention.

US-PAT-NO: 6303346
DOCUMENT-IDENTIFIER: US 6303346 B1
TITLE: Method of producing saccharide preparations
DATE-ISSUED: October 16, 2001
US-CL-CURRENT: 435/96,127/40 ,127/55 ,435/105 ,435/98 ,435/99
APPL-NO: 9/ 632392
DATE FILED: August 4, 2000
PARENT-CASE:
CROSS-REFERENCE TO RELATED APPLICATIONS This application is a continuation of U.S. Ser. No. 09/499,531 filed on Feb. 10, 2000, now U.S. Pat. No. 6,136,571, which is a continuation of U.S. Ser. No. 09/198,672 filed on Nov. 23, 1998, now U.S. Pat. No. 6,129,788, which is a continuation-in-part of U.S. Ser. No. 09/107,657 filed on Jun. 30, 1998, abandoned, which is a continuation-in-part of U.S. Ser. No. 08/979,673 filed on Nov. 26, 1997, the
contents of which are fully incorporated herein by reference.

BSPR:
In another specific aspect, the invention provides a method of **producing a mono
and/or oligosaccharide, such as dextrose, trehalose, isomaltooligosaccaharide, cyclodextrins and maltooligosaccharide preparation, which method comprises an enzymatic** saccharification step, and the subsequent steps of one or more high temperature membrane separation steps and re-circulation of the saccharification enzyme.

BSPR:
Enzymatic processes for producing trehalose from starch or maltooligosaccharides are described by, e.g., Kato et al., (1996), Biosci. Biotech. Biochem., 60 (3), p. 546-550); Kazuhisa et al. (1997), Starch 49, no.
1. p. 26-30; and in EP 764,720.

DEPR:
In the saccharification step, when **producing trehalose, liquefied starch is subjected to the action of an enzyme** capable of first converting maltooligosaccharide (from the liquefaction step) into the non reducing saccharide maltooligosyl trehalose by intramolecular transglycosylation followed by a subsequent step of hydrolyzing the reaction product of the first step (i.e., maltooligosyl trehalose) into trehalose. The saccharification step
may be performed using maltooligosyl trehalose synthase (MTSase) and maltooligosyl trehalose trehalohydrolase (MTHase), e.g., the two enzymes described by Masaru et al. (1996), Biosci. Biotech. Biochem., 60 (3), 546-550). MTSase and MTHase act on amylose or starch to produce trehalose.

DEPR:
Another **enzymatic process for producing trehalose** from starch or maltooligosaccharides (see Kato et al., (1996), Biosci. Biotech. Biochem., 60
(3), p. 546-550) involves using **trehalose-producing enzymes**, a glycosyltransferase and an amylase, respectively, from the hyperthermophilic archae Sulfolobus solfataricus KM1.

DEPR:
Preferably, the saccharification step, when **producing trehalose is performed**

in presence of a MTSase and MTHase, e.g., the enzymes disclosed by Masaru et al. (1996), Biosci. Biotech. Biochem., 60 (3), 546-550).

US-PAT-NO: 6294360
DOCUMENT-IDENTIFIER: US 6294360 B1
TITLE: Saccharide composition containing trehalulose, its preparation and uses
DATE-ISSUED: September 25, 2001
US-CL-CURRENT: 435/100,514/53 ,514/777 ,536/123.13
APPL-NO: 8/ 811003
DATE FILED: March 4, 1997
FOREIGN-APPL-PRIORITY-DATA:
FOREIGN-PRIORITY-APPL-NO: JP 8-070913
FOREIGN-PRIORITY-APPL-DATE: March 4, 1996

BSPR:
The equilibrium point of the reaction system of the maltose/trehalose converting **enzymes inclines to the side of trehalose formation, and the trehalose** yield increases up to an about 80 w/w % (the wording "w/w %" will be abbreviated as "%" unless specified otherwise) when used maltose as a substrate.

BSPR:
The activity of maltose/trehalose converting enzymes used in the present invention is assayed as follows: Add one ml of an enzyme solution to one ml of 10 mM phosphate buffer (pH 7.0) containing 20 w/v % maltose as a substrate, react the mixture at 25.degree., 35.degree. or 60.degree. C. for 60 min, and heat the reaction mixture at 100.degree. C. for 10 min to suspend the enzymatic reaction. Dilute precisely the reaction mixture with 50 mM phosphate buffer (pH 7.5) by 11-fold, add 0.1 ml of a solution containing one unit/ml of trehalase to 0.4 ml of the dilution, incubate the resulting mixture at 45.degree. C. for 120 min, and quantify the glucose content in the mixture using the glucose oxidase method. As a control, use trehalase and an enzyme solution which had been previously inactivated by heating at 100.degree. C. for 10 min, and assay the glucose content similarly as above. With the above assay, determine the content of **trehalose produced by the maltose/trehalose converting enzyme** based on the increased glucose content. One unit activity of the maltose/trehalose converting **enzyme is defined as the amount that forms one pmole trehalose** per minute.

US-PAT-NO: 6284534
DOCUMENT-IDENTIFIER: US 6284534 B1
TITLE: Yeast vector comprising a shortened promoter sequence
DATE-ISSUED: September 4, 2001
US-CL-CURRENT: 435/320.1
APPL-NO: 9/ 242690
DATE FILED: February 23, 1999
PARENT-CASE:
This application is a 371 of PCT/JP97/02924 filed Aug. 22, 1997.

FOREIGN-APPL-PRIORITY-DATA:
FOREIGN-PRIORITY-APPL-NO: JP 8-241062
FOREIGN-PRIORITY-APPL-DATE: August 23, 1996
PCT-DATA:
PCT-DATE-FILED: August 22, 1997
PCT-APPL-NO: PCT/JP97/02924
PCT-371-DATE: February 23, 1999
PCT-102(E)-DATE: February 23, 1999
PCT-PUB-NO: WO98/07873
PCT-PUB-DATE: February 26, 1998
ORPL:
Kobayashi, K., et al., "Gene Analysis of **Trehalose-Producing Enzymes** from Hypertherophilic Archaea in Sulfolobales," Biosci. Biotech. Biochem., vol. 60, No. 10, 1720-1723 (1996).

US-PAT-NO: 6268353
DOCUMENT-IDENTIFIER: US 6268353 B1
TITLE: Method for inhibiting the formation of volatile aldehydes including their related compounds and/or the decomposition of fatty acids including their
related compounds, and uses thereof
DATE-ISSUED: July 31, 2001
US-CL-CURRENT: 514/53,426/321 ,426/331 ,514/23
APPL-NO: 9/ 387520
DATE FILED: September 1, 1999
FOREIGN-APPL-PRIORITY-DATA:
FOREIGN-PRIORITY-APPL-NO: JP 10-249741
FOREIGN-PRIORITY-APPL-DATE: September 3, 1998
DEPR:
Corn starch was prepared into an about 30% suspension which was then subjected to the action of .alpha.-amylase to obtain a liquefied solution with a DE 15. To the solution were added five units/g starch of a **non-reducing saccharide-forming enzyme** as disclosed in Japanese Patent Kokai No. 213,283/95 and 10 units/g starch of a trehalose-releasing enzyme, and 50 units/g starch of
an isoamylase, and the mixture was allowed to react at pH 6.0 and 40.degree. C. for 24 hours. Thereafter, 10 units/g starch of .beta.-amylase was added to the reaction mixture and enzymatically reacted for 10 hours. The resulting mixture was heated to inactivate the remaining enzyme, then in a usual manner decolored, desalted for purification, and concentrated into a syrupy product having a DE of about 38 and a mixture content of about 30% and containing reducing saccharides such as glucose, maltose, and maltotriose along with about
22% trehalose with respect to the syrup. The product is easily handleable and stable at ambient temperature, and it can be arbitrarily used as the captioned product of a syrupy inhibitory agent f or preserving compositions with fatty acids and/or for processing materials thereof.

US-PAT-NO: 6268133
DOCUMENT-IDENTIFIER: US 6268133 B1
TITLE: Method for isolating and recovering target DNA or RNA molecules having a desired nucleotide sequence
DATE-ISSUED: July 31, 2001
US-CL-CURRENT: 435/6,435/91.2 ,536/24.3
APPL-NO: 9/ 103577
DATE FILED: June 24, 1998
PARENT-CASE:
CROSS-REFERENCE TO RELATED PATENT APPLICATIONS This application claims the benefit under 35 U.S.C. .sctn. 119(e) of U.S. Provisional Application No. 60/050,729, filed on Jun. 25, 1997, herein incorporated by reference in its entirety.

ORPL:
Carninci, P. et al., "Thermostabilization and thermoactivation of thermolabile **enzymes by trehalose and its application for the synthesis** of full length cDNA," Proc. Natl. Acad. Sci. USA 95:520-524 (Jan. 1998).

US-PAT-NO: 6232294
DOCUMENT-IDENTIFIER: US 6232294 B1
TITLE: Neuro-function regulatory agent
DATE-ISSUED: May 15, 2001
US-CL-CURRENT: 514/42,514/25 ,514/34
APPL-NO: 9/ 206692
DATE FILED: December 7, 1998
FOREIGN-APPL-PRIORITY-DATA:
FOREIGN-PRIORITY-APPL-NO: JP 9-354068
FOREIGN-PRIORITY-APPL-DATE: December 9, 1997
BSPR:
Trehalose can be produced by various methods. Detailed descriptions of such methods are given up because this invention in itself does not relate to the same. However, considering economical benefit, preferable methods are those which comprise of contacting partial starch hydrolysates with a **non-reducing saccharide-forming enzyme and a trehalose**-releasing enzyme as disclosed in Japanese Patent Kokai Nos. 143,876/95, 213,283/95, 322,883/95, 298,880/95, 66,187/96, 66,188/96, 336,388/96, and 84,586/96. According to these methods, a,.alpha.-trehalose can be produced from starches as costless materials in a relatively-high yield; Examples of commercialized products obtainable thereby are "TREHAOSE.RTM.", a crystalline trehalose powder containing at least 98% trehalose, d.s.b., commercialized by Hayashibara Shoji, Inc., Okayama, Japan; and "TREHASTAR.RTM.", a trehalose syrup containing at least 28% trehalose, d.s.b., commercialized by Hayashibara Shoji, Inc., Okayama, Japan.
.alpha.,.alpha.-Trehalose can be produced by contacting partial starch hydrolysates either with a maltose/trehalose converting enzyme as disclosed in Japanese Patent Kokai Nos. 149,980/96 and 9,986/97 or with conventionally known maltose-and trehalose-phosphorylases in combination.

US-PAT-NO: 6229069
DOCUMENT-IDENTIFIER: US 6229069 B1
TITLE: Method for controlling water content of plant
DATE-ISSUED: May 8, 2001
US-CL-CURRENT: 800/298,435/320.1 ,435/419 ,435/468 ,536/23.6 ,536/24.1
,800/278
,800/295
APPL-NO: 9/ 053702
DATE FILED: April 2, 1998
PARENT-CASE:
This application is a continuation of PCT application no. PCT/JP97/03828 filed on Oct. 23, 1997, which designated the United States and on which priority is claimed under 35 U.S.C. .sctn. 120. This application is also a continuation in part of application Ser. No. 08/736,287 filed on Oct. 24, 1996 ABN, the entire contents of which are hereby incorporated by reference.

BSPR:
To obtain a water-related stress-tolerant plant in the above-mentioned manner, WO 96/00789 and Nature 379 (22) 683-684 (1996) disclose a method for obtaining a plant tolerant to water-related stress by way of controlling the water content of the plant. The method comprises introducing the gene of an **enzyme for trehalose biosynthesis into the plant, thereby to induce the synthesis and accumulation of trehalose** in the plant, and to eliminate the loss in water content by way of the water-retaining effect of trehalose. The disclosure of these references is incorporated herein by reference.